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A PLANT BIOINDICATOR SYSTEM FOR ESTIMATING POLLUTION ABATEMENT --ETC(U)
JAN 82 J M SKELLY, Y YANG DAAG29-78-8-0151

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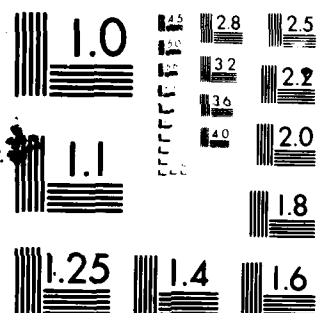
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A PLANT BIOINDICATOR SYSTEM FOR ESTIMATING POLLUTION ABATEMENT -
ELUCIDATION OF THE ULTRASTRUCTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL
BASIS OF SENSITIVE AND TOLERANCE TO NO₂, SO₂, AND O₃

FINAL REPORT

Dr. John M. Skelly

Dr. Yaw-Shing Yang

January 11, 1982

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Statement of the Problem Studied

The specific objectives of this research are:

- 1) to compare the stomatal frequency of two selected eastern white pine, one sensitive and one tolerant to O_3 ;
- 2) to characterize the fine structure of the epicuticular wax on needle surface and to compare the wax structure of the two selected clones;
- 3) to continue the determination of the relative sensitivity of selected eastern white clones to NO_2 , SO_2 , and O_3 used singly and in combination;
- 4) to investigate photosynthetic and respiratory rates of different eastern white pine clones during light and dark conditions as influenced by NO_2 , SO_2 , and O_3 ; and
- 5) to determine the influence of NO_2 , SO_2 , and O_3 fumigation on needle chlorophyll content.

Summary of the Most Important Results

Ten clones of eastern white pine (Pinus strobus L.) representing three pollutant sensitivity classes (sensitive, intermediate, and tolerant) were collected from within the boundary of the Radford Army Ammunition Plant (RAAP), Radford, Virginia and exposed to 0.00 - 0.30 parts per million (ppm, V/V) of nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3), singly and in combination, during various fumigation studies. The different sensitivities of eastern white pine clones to ambient pollutants as observed at the RAAP was elucidated based on the clonal ultrastructures and clonal physiological and biochemical performances during controlled pollutant exposures. After exposure to 0, 0.10, and 0.30 ppm O_3 for 6 h/day for 7 consecutive days. Needle alkane concentration of an ozone-tolerant clone was found significantly greater ($P=0.05$) than that of a selected ozone-sensitive clone as expressed on both a fascicle fresh weight and fascicle surface area basis. However, the fine structure of epicuticular wax exudated on needle surfaces of both clones was characterized by a fibrillar or an amorphous, plate-like wax structure regardless of clonal ozone sensitivity or the ozone concentration used in the fumigation. Stomatal frequency on a per unit area basis was not significantly different between the above two clones. It was concluded from ultrastructural studies that alkane content of epicuticular wax of white pine needles is related to clonal O_3 sensitivity.

By use of vegetatively propagated ramets, white pine responses to pollutant exposure were found to be genetically inherited in all test clones with various degrees of repeatability. The types of foliar injury induced

by NO_2 , SO_2 , or O_3 were not distinguishable among the pollutant treatments. The predominant visible symptoms resulting from pollutant exposures included general chlorosis, pigmented mottling or necrotic lesions. The amount of foliar surface symptomatic in each clone was not always proportional to pollutant dosage (dose x duration). Foliar symptom expression is thus considered not to be a reliable indexing parameter to determine the actual exact exposure dose of individual or combinations of the gaseous pollutants tested. When grown in pollutant-free environments, the elongation of current year needles of white pine clones was dependant upon clonal genotype and environmental conditions. When exposed to pollutants, the suppression of needle elongation was more evident in sensitive clones than in tolerant clones. Such length reduction was especially significant with high pollutant concentrations. However, the final length of current year needles was not usually consistant with clonal sensitivity to pollutant type or pollutant dosage. Good agreement was found between the degree of reductions in needle net photosynthesis rate, needle dry weight, needle chlorophyll content and the ranking of clonal sensitivity to pollutant regardless of pollutant species and/or exposure dose. The sensitive clones exhibited the greatest reduction of net photosynthesis resulting from pollutant exposure and was followed by intermediate and tolerant clones. In the sensitive clones, all of the pollutant-treated trees had less biomass production (based on unit needle length basis) than control plants. None of the plants of the tolerant clone were observed to have produced different biomass ($P=0.05$) from control plants after they had been exposed to the various pollutants. Both chlorophyll a and chlorophyll b content of current year needles of all test clones were reduced due to pollutant exposures with the sensitive clone being suppressed the most. The amount of chlorophyll reduction was

a function of pollutant species, pollutant concentration, and white pine clone. Results indicate that the relative sensitivity of eastern white pine clones to NO_2 , SO_2 , and O_3 could be objectively determined by evaluating the performance of various plant responses. Since plant reaction to pollutants are genetically controlled, this study also has suggested that the presence of NO_2 , SO_2 , or O_3 in the ambient air surrounding the RAAP can be qualitatively and quantitatively determined by using the cross-examination of the response of a collection of sensitive indicators. Such techniques may provide a reasonable tool for differentiating the existence and the concentration of certain pollutants through evaluating specific types of plant response.

LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP
DURING THIS PERIOD, INCLUDING JOURNAL REFERENCES:

1. Skelly, J. M. and Y. S. Yang. 1980. Growth impact of a periodic source of industrial air pollutant emission on eastern white pine: an update and re-evaluation of previous reports. *Phytopathology* 80:693. (Abstract).
2. Trimble, J. L. and D. M. Orcutt. 1980. Alkane content of the epicuticular wax of two Pinus strobus L. clones differing in sensitivity to ozone. *Phytopathology* 80:693. (Abstract).
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4. Yang, Y. S. 1980. Effects of ozone, sulfur dioxide and nitrogen oxides on the growth of eastern white pine. *Phytopathology* 70:694. (Abstract).
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6. Yang, Y. S., J. M. Skelly, and B. I. Chevone. 1982. Clonal response of eastern white pine to O₃, SO₂, and NO₂ exposure singly, and in combination. *Environmental Pollution* (in revision).
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8. Yang, Y. S., J. M. Skelly, B. I. Chevone, and J. B. Birch. 1982. Effects of ozone on photosynthesis, transpiration, and dark respiration of three eastern white pine clones. *Phytopathology* (in revision).

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tivity to ozone, sulfur dioxide, and nitrogen dioxide. pp.159

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Thesis Title: Epicuticular wax and stomata characterization
of two Pinus strobus L. clones differing in sensit' ity to
ozone. pp. 79

VARIATION IN THE PHYSIOLOGICAL PROCESSES OF
EASTERN WHITE PINE (PINUS STROBUS L.)
DIFFERING IN SENSITIVITY TO
OZONE, SULFUR DIOXIDE, AND NITROGEN DIOXIDE

Yaw-Shing Yang

ABSTRACT

Ten clones of eastern white pine (Pinus strobus L.) representing three differing pollutant sensitivity classes (i.e. sensitive, intermediate, and tolerant) were exposed to ozone, sulfur dioxide, and nitrogen dioxide singly, and in combinations at various concentrations. Visible symptom expression, needle length, and needle dry weight of current year needles were determined weekly during long-term pollutant exposures. One clone of each sensitive class was selected to study the effects of long-term exposures with ozone, sulfur dioxide, or nitrogen dioxide on photosynthesis, transpiration, and dark respiration.

Genetically controlled plant responses to pollutant exposures were found in all clones with various degrees of repeatability. A general plant sensitivity to all of the pollutants was not observed in test clones. Foliar symptom expression was only categorically differentiated among sensitivity classes but was not distinguishable in clonal response within the same class. At the end of long-term exposures, needle length was not significantly different among sensitive, intermediate, and tolerant classes nor among pollutant treatments. Good agreement was found among white pine gas exchange rates, needle dry weight, and chlorophyll content with respect

Ph.D. Dissertation

to clonal sensitivity. Sensitive clone exhibited the greatest reduction in net photosynthesis due to ozone and sulfur dioxide exposures followed by intermediate and tolerant clones. Early stimulation of dark respiration was induced by ozone and sulfur dioxide exposures in sensitive clone followed by a dramatic decrease at late stages of long-term experiment. Nitrogen dioxide at test concentrations did not significantly reduce net photosynthesis, transpiration, and dark respiration rates. There was a correlation between clonal needle dry weight, chlorophyll content, and degree of its pollutant injury. Different modes of injury actions by different pollutants are proposed based upon presented data.

Results support the concept that the ranking of plant sensitivity to pollutants could be varied with plant response chosen as indexing criterion. Presented data suggest that the adverse effects of pollutant exposures on white pine growth are primarily due to inhibition of net photosynthesis, less chlorophyll content, and high respiration rate. Visible injury and needle length are concluded to be less superior than net photosynthesis, transpiration, dark respiration, chlorophyll content, and needle dry weight measurement in providing reliable indexing parameter for white pine pollution sensitivity prediction.

EPICUTICULAR WAX AND STOMATA CHARACTERIZATION
OF TWO PINUS STROBUS L. CLONES DIFFERING
IN SENSITIVITY TO OZONE

Judy Lynn Trimble

ABSTRACT

Two clones of eastern white pine (Pinus strobus L.), one sensitive and one tolerant to O_3 , were exposed to 0, 10, and 30 pphm O_3 6 h/day for 7 consecutive days. Intact fascicles were excised from the terminal apex of each tree at 6 h and at 7 days after initiation of fumigation. The fine structure of the epicuticular wax was examined with the scanning electron microscope (SEM). All samples were characterized by a fibrillar wax structure or an amorphous, plate-like wax structure, regardless of O_3 exposure or O_3 sensitivity. Stomatal frequency was determined by SEM examination of needles from which all surface wax and debris had been removed by dipping the needles in chloroform. Stomatal frequency per unit area was not significantly different between the two clones. Additional fascicles were harvested from those trees exposed to 0 and 30 pphm O_3 at 10 wk and at 12 wk after fumigation. The epicuticular wax extracted from these fascicles was analyzed for alkane content using gas liquid chromatography. Hentriacontane (C_{31}) and tritriacontane (C_{33}) were the predominant alkanes. Alkane concentration was significantly greater for the tolerant clone on both a fascicle surface area and fascicle weight basis regardless of exposure. In both clones exposure to O_3 resulted in higher alkane concentration through an undetermined mechanism. It was concluded that the alkane content of epicuticular wax is related to O_3 sensitivity of eastern white pine.

CLONAL RESPONSE OF EASTERN WHITE PINE TO
 O_3 , SO_2 , AND NO_2 EXPOSURE, SINGLY AND IN COMBINATION

Yaw-Shing Yang, John M. Skelly and Boris I. Chevone

ABSTRACT

Eight clones of eastern white pine (Pinus strobus L.) in three pollutant sensitivity groups (sensitive, intermediate and tolerant) were exposed to 0.05 and 0.10 ppm of oxone, sulfur dioxide and/or nitrogen dioxide 4 h daily for 35 consecutive days. Visible symptoms, length and dry weight of first year needles were determined to evaluate clonal response to these pollutants. At the time of exposure, first year needles were 21 to 25 days old. A general sensitivity by white pine sensitivity group to all three pollutants, singly and in combination, was not observed. The sensitivity of the clones was dependent on the type of pollutant, its concentration and the plant response used as the indexing factor. Foliar injury was categorically differentiable among the three sensitivity groups but varied within the same group. Needle length was an inconclusive measure of clonal pollutant sensitivity. Needle dry weight was the parameter best associated with clonal pollutant sensitivity. These results demonstrate the limited value of research reports in which a single plant response parameter was used to determine the sensitivity of plants to air pollutants.

Submitted to Phytopathology

EFFECTS OF OZONE ON PHOTOSYNTHESIS, TRANSPIRATION AND DARK
RESPIRATION OF THREE EASTERN WHITE PINE CLONES

Y. S. Yang, J. M. Skelly, B. I. Chevone, and J. B. Birch

ABSTRACT

Net photosynthesis, transpiration and dark respiration rates during O_3 exposure were determined in three clones of 2-yr-old grafted eastern white pine (*Pinus strobus* L.) differing in sensitivity to O_3 . Fumigations occurred for 4 h daily with 0.10, 0.20, and 0.30 ppm (v/v) O_3 for 50 consecutive days. Photosynthesis and transpiration rates were determined simultaneously with an infrared dual-gas analyzer during daily O_3 exposure. Dark respiration was measured 4 h after daily O_3 fumigation for 4 consecutive h. After 4 h O_3 exposure at 0.30 ppm, the inhibition of net photosynthesis was 21%, 12%, and 7% for the sensitive, intermediate and tolerant clone, respectively. Photo-transpiration was inhibited in sensitive and tolerant clones but stimulated in the clone with intermediate sensitivity to O_3 . Recovery of gas exchange rates one h after the termination of ozonation occurred with the degree of recovery depending upon the clonal sensitivity and the O_3 concentration. Dark respiration increased in the O_3 sensitive clone only and dark transpiration was unaffected in all three clones after 4 h exposure to 0.30 ppm O_3 . The implications of photosynthetic inhibition and stimulation of dark respiration due to O_3 fumigation in relation to clonal sensitivity to O_3 are discussed.

Submitted to Phytopathology

CHEMICAL AND STRUCTURAL CHARACTERIZATION OF THE NEEDLE
EPICUTICULAR WAX OF TWO PINUS STROBUS L. CLONES DIFFERING
IN SENSITIVITY TO OZONE

J. L. Trimble, J. M. Skelly, S. A. Tolin and D. M. Orcutt
1982 Accepted for Publication Phytopathology 72:

ABSTRACT

Two clones of eastern white pine (Pinus strobus L.), one sensitive and one tolerant to ozone (O_3), were exposed to 0.0 and 0.30 ppm O_3 for 6 hr/day for 7 consecutive days. Intact fascicles were excised from the terminal apex of each tree, and fine structure of epicuticular wax was examined by scanning electron microscopy. All samples were characterized by a fiberillar wax structure or an amorphous, plate-like wax structure, regardless of O_3 exposure or previously determined O_3 sensitivity. Epicuticular wax removed from harvested fascicles was analyzed for alkane content using gas-liquid chromatography. Hentriacontane (C_{31}) was the predominant alkane concentration was significantly greater for the tolerant clone on both a fascicle surface area and fascicle weight basis, regardless of exposure.

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